SHRI MATHURADAS MOHOTA COLLEGE OF SCIENCE, NAGPUR

ESTIMATION OF CALCIUM IN DIFFERENT MILK SAMPLES FROM NAGPUR, MAHARASHTRA

PROJECT REPORT

Submitted to RTM Nagpur University for the fulfilment of Master of Science Degree for the academic year 2019 – 2020

Submitted By

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M.Sc. – (chemistry) semester – IV

Under the supervision of

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CERTIFICATE

This is to Certify that **Ms. Ashwini L. Dekate** has carried out the work presented in this project report entitled " **ESTIMATION OF CALCIUM IN DIFFERENT MILK SAMPLES FROM NAGPUR, MAHARASHTRA"** under my supervision in the post graduate department of Chemistry, **SHRI MATHURADAS MOHOTA COLLEGE OF SCIENCE, NAGPUR** during the academic year 2019 – 2020 in partial fulfilment of the requirements prescribed for the Degree of Master of Science (chemistry) of R. T. M Nagpur University.

This work has not been submitted anywhere for degree, diploma and certificate to any other University.

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DECLARATION

The author hereby declares that, tu work presented in this project entitled **" ESTIMATION OF CALCIUM IN DIFFERENT MILK SAMPLES FROM NAGPUR, MAHARASHTRA"** has been carried out by the candidate under the guidance of **Dr. C. B. Talwtkar** The work has been carried at **SHRI MATHURADAS MOHOTA COLLEGE OF SCIENCE NAGPUR** and has been submitted as the project work for degree of Master Of Science in Chemistry. The result and conclusion given in the project work have been arrived as a consequence of the research work carried out by the candidate.

Ms. Ashwini L. Dekate

Date :

Guide :

Dr. C. B. Talwtkar

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Place : Nagpur

Date :

Ms. Ashwini L. Dekate

M. Sc. (Sem-IV)

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CHAPTER 1

INTRODUCTION

Introduction

What is milk ?

Milk is a highly nutritious food and ideal for microbial growth. The fresh milk deteriorates easily to become unsuitable for processing and human consumption.

Milk obtained from healthy animal's udder is free from pathogenic bacteria but some of the animals in field condition may he suffering from sub-clinical mastitis and are excreting the causative agent in milk, such milk contaminates the bulk milk. Moreover, fresh milk may get microbial contamination from utensils, animal skin, environment , or water user for cleaning etc.

Foodborne disease are a common and widespread global problem. Several outbreaks have been reported as a result of consuming contaminated milk that may look, taste and smell perfectly normal but are in fact contaminated with large number of harmful bacteria.

Milk contaminated by high levels of spoilage bacteria usually becomes unsuitable for further processing since it does not meet the consumers expectations in terms of health (nutritional value), safety (hygienic quality) and satisfaction (sensory attributes).

The presence of Califorms in good of animal origin indicates environmental and fecal contamination since these micro-organisms are abundant in the environment food.

E. Coli is important as mastitis pathogens and widely distributed in the farm environment.

Among the coliforms, Escherichia coli organisms are the most common contaminants of raw and processed milk.

It is a reliable indicator of fecal contamination of water and food such as milk and dairy products.

Staphylococcus aureus is one of the important cause of food-borne diseases in humans, S. Aureus is commonly associated with intoxication due to its ability to produce a variety of potent enterotoxin.

Identical S. Aureus strains have occasionally been isolated from dairy cows and hands of milking person (9) , but strings originating from bovine mastitis in general represent a genetically different cluster than the human strains, suggesting host specificity.

Milk is very important due to its special nutritive value and important role for human and animal health . It has all the substances needed by organisms in its easiest assimilable form. It has high value protein (casein, lactalbumine and lactoglobuline providing essential amino acids), fat providing energy (9.3 kcal /g), a low melting point (29-34 0c), small globules stimulating an easy assimilation, A and D vitamins playing an special role in Calcium and phosphorus fixation in bones, low cholesterol compared to other food animal origin (fresh milk 10, skimmed milk 3 , butter 280, fat cheese 150-200, pork 100-130, egg yolk 1400 mg/100 g product). Milk or Milk sugar, due to the bacteria living in the intestine is transformed in lactic acid with a benefit influence upon our body. Minerals are also very important (Calcium , phosphorus etc.). Due to its nutritive value , milk is recommended to young and old people, being considered a complete food. The nutritive and energetic value of one milk kilogram corresponds to the ones of 0.5 Kg beef or 8-9 eggs. One kilogram of milk contains : water 84-90% , fat 2-6%, Protein 3-4%, lactone 4-5%, minerals 1% and supplies about 668 Kcal. Therefore , milk is a very important raw material for food industry.

Goats form an important component of livestock industry and play a vital role in the socio-economic structure of rural poor. India has the second highest goat population in the world which is round 120 million constituting about 15% of world's goat population only next to china.

Goats produce about 2% of the world’s total annual milk supply.

Dairy goats in their prime (generally around the third or fourth lactation cycle) average 6 to 8 lb (2.7 to 3.6 kg) of milk production daily (roughly 3 to 4 US quarts (2.7 to 3.6 liters)) during a ten-month lactation, producing more just after freshning and gradually dropping in production towards the end of their lactation. The Milk generally averages 3.5% butterfat.

There is accumulating evidence that a high protein intake increases weight loss and prevents weight gain.

The large multicenter European study Diet, obesity and Genes (Diogenes) have recently shown that even a modest increase in protein intake combined with a modest reduction in glycemic index can prevent weight regain after a weight loss and reduce the prevalence of overweight among children.

The beneficial effect of a high protein intake seems to be due to a higher diet-induced thermogenesis (DIT) , increased satiety and decreased hunger.

It has been speculated that different protein sources may affect satiety and DIT differently, but only very limited data from human studies on this topic is available.

Dairy products are rich in protein. Milk contains 32 g protein per litre, the protein content consisting of 80% casein, unlike whey, coagulates in the stomach, owing to its precipitation by gastric acid.

Therefore, the gastric emptying time for casein is delayed compared with whey, resulting in a slower release and absorption of amino acids from casein.

In 1997, Boirie et.al. introduced the concept 'slow' and fast protein to describe these differences in digestion and absorption of whey and casein. It has been suggested that a fast protein like may be more satiating than a slow protein like casein. However, data from studies examining this is inconclusive.

Hall et al.that a preload drink of whey was more satiating than an is energetic casein drink, and that it increased postprandial cholecystokinin (CCK) and glucagon-like peptide (GLP)-1 response more than the casein drink, indicating that CCK and GLP-1 may be mediators of the increased satiety response to whey.

Interestingly, Diepvens et al. Found that the unique combination of whey and casein found in Milk stimulates CCK and GLP-1 more than whey alone. Others have reported no difference in the effect of whey and casein on satiety.

Whilst some have reported that casein is more satiating than whey.

In the past 20 years there has been a rapid growth in organic products market. The loss of faith traditional (conventional) products, resulting from the detection of contaminants, pesticides, toxins etc. There in promotes this situation. In addition, the idea of healthy diet and environment protection, which are applicable to organic agriculture, are gaining popularity among consumers. Thus in 2014 the European market turnover of organic products reached 26.2 billion, more than the previous year by 7.6% . In particular, in Germany the turnover amounted to 7.91 billion, in France -4.83 billion. In the US the turnover has reached 27.06% billion.

In Ukraine, the organic products market has been growing since the early 2000's and is increasing annually. According to the Swiss-Ukrainian project FIBL, Ukraine occupies the 11^{th} place in Europe in terms of organic food production and by 2020 may become one of the five largest organic producers in the world. In 2014 in Ukraine 400,764 hectares were farmed organically, there were about 250 organic products producers and processors, and the internal market for organic products amounted to 14.5 million.

Despite the increase in the number of organic farms, which are certified according to respective requirements, comparative studies on the key indicators of conventional and organic products (e.g. milk and dairy products) are absent in Ukraine. Many of foreign works slow the change of fatty acid composition of milk, depending on dairy management type. It is known that organic milk contains higher content of -3 fatty acids, alpha-linolenic acid, alpha-tocopherol and Fe compared to conventional milk.

However, there are significant differences in studies results, depending on the country of origin, animal's breed, season, diet etc. The comparison of the data on the concentrations of PUFA, -3acids and CLA in organic and conventional cow's milk in different countries or geographic regions showed significant differences, and in some cases contradicting data has also been obtained in studies within the same country.

At the same time, there are still less studies on the content of dry matter, total protein and other physical and chemical parameters of organic milk and the results are extremely ambiguous.

Milk has long been recognized as a valuable food of pastoralist diets in all the world, also it is a nutrient food and is recognized to contribute a high proportion of the nutrients, such as micronutrients, in clouds calcium, phosphorus, vitamins like B and D, high quality protein such as casein protein, also fatty acid composition of milk fat has relation to its potential health benefit and impact on the human health.

Different factors, such as race of cows, genetic variants, stage of lactation and environmental factors which can significantly affect on milk component and properties of milk.

focused of global warming may be felt in a high variety of economic, social, and environmental sectors, including marine life, energy usage, forestry, water resources and human health, rangeland ecosystems, etc. Today's considerable research has show been directed at an assessment of the effect of climate on most of these sectors.

Calcium

What is calcium ?

Chemical element calcium is essential for living organisms, including humans. It is the most abundant mineral in the body and vital for good health.

We need to consume a certain amount of calcium to build and maintain strong bone and healthy communication between the brain and other part of the body. Calcium is found naturally in many foods. It is also added to certain product and supplement are available.

Calcium is the most abundant metal and the fifth-most abundant element in the human body. As electrolytes, calcium ions play a vital role in the physiological and biochemical process of organisms and cells : in signal transduction pathways where they act as a second messenger, in neurotransmitter release from neurons; in contraction of all muscle cell types; as cofactors in many enzymes; and in fertilization. Calcium ions outside cell are important for maintaining the potential difference across excitable cell membranes as well as proper bone formation.

Why calcium is important ?

Calcium is important for overall health. Almost every cell in over body uses calcium in some way. Our bones store calcium in addition to providing support for our bodies. When our body absorb less and less calcium from our diet, it become calcium deficient. Over time this process can cause or contribute to osteopenia or osteoporosis.

We get calcium from food we eat. Calcium rich food include milk cheeses and other dairy products we can also get calcium from vitamin and supplements.

In animals with skeletons mineralized with calcium isotopic composition of soft tissues reflects the relative rate of formation and dissolution of skeletal mineral. In humans, changes in the calcium isotopic composition of urine have been shown to be related to changes in bone mineral balance.

When the rate of bone formation exceeds the rate of bone resorption, the 44Ca/40Ca ratio in soft tissue rises and vice versa. Because of this relationship, calcium isotopic measurements of urine or blood may be useful in the early detection of metabolic bone diseases like osteoporosis.

Function

Calcium is an essential element needed in large quantities. The Ca2+ ion acts as an electrolyte and is vital to the health of the muscular, circulatory, and digestive systems; is indispensable to the building of bone; and supports synthesis and function of blood cells. For example, it regulates the concentration of muscles, nerve conduction, and the clotting of blood. As a result, intra- cellular and extracellular calcium levels are tightly regulated by the body. Calcium can play this role becuase the Ca2+ ion forms stable coordination complexes with may organic compounds , especially proteins; it also forms compounds with a wide range of solubility 's , enabling the formation of the skeleton.

Normal limits

Our bodies like to keep the amount of calcium in our blood within a certain narrow range. This range allows the same in our body to stay healthy and perform jobs necessary for life.

Total calcium references range in males is as follows

- Younger than 12 months: not established
- Age 1-14years: 9.5-10.6 mg/dL
- Age 15-16 years: 9.5-10.5 mg /dL
- Age 17-18 years: 9.5-10.4 mg/dL
- Age 19-21 years and older : 8.9-10.1 mg/dL

Total calcium references range in females is as follows

- Younger than 12 months : not established
- Age 1-11 year : 9.6-10.6 mg/dL
- Age 12-14 years : 9.5-10.4 mg/dL
- Age 15-18 years : 9.1-10.3 mg/dL
- Age 19 years and older : 8.9-10.1mg/dL

Hypocalcaemia

Hypocalcaemia, commonly known as calcium deficiency disease, occur when calcium levels in the blood are low, along term deficiency can lead to dental changes, cataracts alterations in the

brain and osteoporosis which cause the bones to become brittle. Complication hypocalcaemia can be like threatening, and if the condition these untreated, it could eventually lead to death.

Hypercalcemia

Excess intake of calcium may cause hypercalcemia. However , because calcium is absorbed rather inefficiently by the intestines, high serum calcium is more likely caused by excessive secretion of parathyroid hormone (PTH) or possibly by excessive intake of vitamin D, both which facilitate calcium absorption. It may also be due to bone destruction that occurs when tumours metastasize within bone. All these conditions result in excess calcium salts being deposited in the heart, blood vessels, or kidneys. Symptoms include anorexia, nausea, vomiting, memory loss, confusion, muscle weakness, increased urination, dehydration, and metabolic bone diseases. Chronic hypercalcemia typically leads to calcification of soft tissue and it's serious consequences : for example, calcification can cause loss of elasticity of vascular walls and disruption of laminar blood flow and then to plaque rupture and thrombosis.

Hypercalcemia is a condition in which the calcium level in yours blood is above normal. Too much calcium in your blood can weaken your bones, create kidney stones, and interfere with how your heart and brain work.

Hypercalcemia is usually result of overactive parathyroid glands this four tiny glands are situated behind the thyroid gland other causes of hypercalcemia include certain medical disorder, some medications and taking too much of calcium and vitamin D supplements.

A calcium deficiency may have no early symptoms. To avoid complication, a person should seek prompt diagnosis and treatment if they experience any of the system listed below.

What are the symptoms?

The symptom described below may become worse as the disease progresses.

Muscles aches, cramps and spasms are earliest signs of a calcium deficiency. People tend to feel pain in the thighs and arms, particularly the underarms, when walking and otherwise moving.

A calcium deficiency can also cause numbness and tingling in the hands arms feet, legs, around mouth.

Neuromuscular excitability, which potentially cause tetany and disruption of conductivity in cardiac tissue.

Bone disease

As calcium is heavily involved in bone manufacture, many Bone disease can be traced to problems with the organic matrix or the hydroxyapatite in molecular structure or organization. For example, osteoporosis is a reduction in mineral content of bone per unit volume, and can be treated by supplementation of calcium, vitamin D, and bisphosphates. Calcium supplements may benefit the serum lipid in women who have passed menopause as well as older men; in post-menopausal women calcium supplementation as also appears to be inversely correlated with cardiovascular

disease. Inadequate amounts of calcium, vitamin D, or phosphates can lead to the softening of bones, known as osteomalacia.

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CHAPTER 2

REVIEW OF LITERATURE

Review of literature

Theresa M Smith et Al (1985) In this work, An increased prevalence of osteoporosis had been observed in lactase -deficient subjects. This association had been attributed to an avoidance of calcium-containing dairy products by lactase deficient subjects and/or an adverse affect of lactose malabsorption on calcium absorption. Because the lactose in yogurt can be digested and absorbed by hypotactic subjects, They tested of lactase-deficient subjects and controls to absorb calcium from milk and yogurt. Subjects ingested 270 mg of Ca plus 45 Ca in 250 g of milk or 147 g of commercial, unflavoured yogurt and blood radioactivity was assessed at intervals over 24hr. Based on the areas under the blood radioactivity curves, lactase-deficient subjects absorbed 45Ca from both sources at least as well as did the controls. While we found no evidence to indicate that calcium in yogurt is better absorbed than calcium in milk, yogurt remains an excellent source of calcium because this fermented product was well tolerated by lactase-deficient subjects.

T Powell Gaines al (1986) They had studied the bioavailability of zinc and calcium from human, cow's, goat, and sleep milk was evaluated by an in vitro method (1995) that involves a stimulated human gastrointestinal digestion followed by measurement of dialyzability of zinc and calcium. Zinc availability of milk showed the highest value for human milk (15.0%) and the lowest for sheep milk (1.0%), in both whole and skim milk. Calcium availability of the different types of milk did not differ significantly and ranged between 18 and 23%. No significant differences in availability between the whole and skim were found for both elements, except for zinc in cow's milk.

Lihua Shen Harry et al (1990) They had studied calcium and vitamin D, milk composition and a prospective study among postmenopausal women (2003). Short trails of calcium supplementation show that it reduces loss of bone density in postmenopausal women; longer observational studies did not generally find a lower risk of hip fracture with higher-calcium diets. Fewer studies have focused on vitamin D in preventing postmenopausal osteoporosis or fractures: they assessed relations between postmenopausal hip fracture risk and calcium, vitamin D, and milk consumption.

In an 18 -y prospective analysis in 72, 337 postmenopausal women, dietary intake and nutritional supplement use were assessed at baseline in 1980 and update several times during follow-up. We identified 603 incident hip fractures from low or moderate trauma. Relative risks (RRs) from proportional hazards models were controlled for other dietary and non literary factors : Women consuming >12.5 ug vitamin D/d from food plus supplement had a 37% lower risk of hip fracture (RR = 0.63; 95% CI:0.42, 0.94) than did women consuming < 3.5ug/d. Total calcium intake was not associated with hip fracture risk (RR=0.96; 95% CI: 0.68, 1.34 for > 1200 compared with < 600 mg/d. Milk consumption was also not associated with a lower risk of hip fracture (P for trend =0.21).: An adequate vitamin D intake was associated with a lower risk of osteoporotic hip fracture in postmenopausal women. Neither milk not a high-calcium diet appears to reduce risk. Because women commonly consume less than the recommended intake of vitamin D, supplement use or dark fish consumption may be prudent.

Diane Fresknich, Walter C Willette et Al (1996) They had studied detection of calcium based neutralizers in milk and milk products by AAS. (2013). Current study was carried out with the intent to standardize detection and estimation method for calcium (Ca) based neutralizers in milk and milk based indigenous products (khoa and paneer) using atomic absorption spectroscopy (AAS). Neutralized milk, khoa and paneer samples were prepared using milk with developed acidity to which calculated quantity of neutralizer (Ca based) was added. Rosalic acid test results get masked at times due to developed acidity which neutralizes the alkalinity imparted by neutralizer and hence gives false result with time in neutralizers added samples. Atomic absorption spectroscopy proved to be an accurate estimate which could detect the abnormal rise in mineral concentration even with slight addition of neutralizers in comparison with control milk and milk products. Formalin, which was a commonly used preservative in milk samples for chemical analysis, did not have any significant impact on estimation of calcium in the neutralized milk during storage.

Shrihari Ashok Pingle et Al (2002) They had performed the titretric estimation of calcium from different milk. (2006). Milk is primary source of nutrition. It contains dissolved carbohydrate, protein, vitamins, fats, minerals like calcium, magnesium , and sodium. Calcium is the most abundant mineral in human body. It is important for biological processes. The aim of the present study was to determine the amount of calcium in milk samples from different sources and the effect of heat on calcium content. Milk samples were collected from Desi cow, Jersey cow, Goat, Buffalo, and pasteurized milk. Amount of calcium was determined by EDTA titration method. The amount of calcium was highest in goat milk and lowest pasteurized packed milk.

M.B. Petrovich et Al (2007) A flow-injection system with sample and reagent addition by the synchronous merging zoned approach for calcium determination in milk by flame AAS is proposed. Main parameters were optimized using a factorial design with central point. The optimum conditions were 2.5% (m/v) for La concentration, 8 mL min-1 for the carrier flow-rate, 20 cm for coiled reactor and 250 mL for sample volume. Different sample preparation procedures were evaluated such as dilution in water or acid and microwave assisted decomposition using concentrated or diluted acid. The optimized flow system was applied to determine CA in eleven commercial milk samples and two standard reference materials diluted in water. Similar calcium levels were encountered comparing the results obtained by the proposed method (dilution in water) with those obtained using microwave oven digestion. Results obtained in two standard reference materials were in agreement at 95% confidence level with those certified. Recoveries of spiked samples were 93%-116% range. Relative standard deviation (n=12) was < 5.4% and the sample throughout was 150 measurement per hour, corresponding to a consumption of 250uL of sample and 6.25 mg La per determination.

R.M. Daly et Al (2009) Some epidemiology and clinical studies have shown that increased dairy consumption or calcium and vitamin D supplementation can have a beneficial effect on blood pressure, and lipid and lipoprotein concentrations. The aim of this study was to access the long term effects of calcium-vitamin D3 fortified milk on blood pressure and lipid lipoprotein concentrations in community dwelling older men. This is a sub study of a 2 year randomized controlled trial in which 167 men aged 450 years were assigned to receive either 400 ml per dya of reduced fat (B1%) milk fortified with approximately 1000 mg of calcium and 800 IU of vitamin D3 or to a control group receiving no additional fortified milk. Weight, blood pressure, lipid and lipoprotein concentrations were measured every 6 months.

R. Sowmya et al (2013) The objective of the study was to analyse the consumption of dairy products and dietary calcium by women in the context of bone mineral density and to assess opportunities to prevent osteoporosis in a dietary manner. The study was carried out with 712 polish women. In 170 women aged 32 to 59 bone mineral density was measured. The data on the consumption of dairy products and dietary calcium and some other osteoporosis risk factors was collected from 712 women. The average calcium intake from a diet was 507 mg/day. Only 2% of the women met polish calcium intake recommendations. During adulthood, dairy product consumption or dietary calcium intake did not differ significantly between women with low BMD(below-1SD) and women with regular BMD (>-1SD) (47.4 vs. 44.3 servings/week and 459 vs. 510 mg/day, respectively, p>0.05). The odds ratios adjusted for age, menstruations and BMI

in women with upper BMD tercile in comparison to the reference group (bottom tercile) was 2.73 (95%Cl : 1.14, 6.55; $p<0.05$) for the daily consumption of dairy products during the pre-school period and 2.40 (95% CI : 1.01, 5. 70; $p<0.05$) for the daily consumption of dairy products during the school period. Two clusters of women were established. In the S1 cluster, low BMD(below-1SD) was associated with older age (>50 years), lack of menstrual cycle. In the S2 cluster, regular BMD (>1SD) was related to younger aged women (<50years), presence of menstrual cycle, consumption of higher level of dairy products (>28 servings/week) during adulthood and daily intake of diary products during childhood and adolescence.

Lidia wadolowska Al(2015) have estimated the magnesium and calcium from fresh milk(2015). Four different fresh milk were collected from local market, knowing the benefits of heavy metals present in fresh milk, the aim of this research is to determine the heavy metals like magnesium and calcium present in fresh milk samples from Nashik District region. The estimation of magnesium and calcium is done by simple method.i.e. volumetrically, and the results tab M Dated.

Dr. Khailas kapadia et al (2015) have studied the determination of calcium and phosphorus in milk (1990). A fast, accurate method for determining CA and P in milk was developed. A 1-ml sample of milk was ashed without pre-drying for 1 h and the diluted HCl extract was used for the determination of Ca by atomic absorption spectroscopy and P by colorimetric with measurement of the phosphomolybdenum blue complex at 880 nm for maximum sensitivity. Results were in good agreement with those found for 2hr ashing with or without the preliminary step for evaporating to dryness prior to ashing. Recovery ranged from 101.3 to 102.0% for CA and 99.1%to 100.1%for p when 1ml sample of milk was spiked with a 1ml aliquot of a CaHPO4 standard solution and analysed by the method. The method had a precision of approximately 1.0% coefficient of variation for both CA and P in milk and in the CaHPO4 standard solution

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CHAPTER 3

MATERIAL METHOD

Method Material

Collection of milk samples

Different milk samples were obtained from the animals viz. Cow, Goat, and Buffalo commercially.

Available packaged milk samples (Amul, Haldiram, Dinshaw, Gowardhan, Mother dairy, and Nestle milk powder, Britannia milk powder) were obtained from Nagpur district, Maharashtra.

The fresh milk samples were collected in properly washed and cleaned bottles. All milk samples were brought to laboratory for estimating amount of calcium

Estimation of calcium from different milk samples

Material required

EDTA solution (0.01 M), $ZnSO_4$ solution (0.01 M), (NH_4Cl +NH_4OH) buffer solution pH 10, Eriochrome black-T (EBT), burette, volumetric flask 100ml, conical flask-250 ml, beakers, pipette, measuring cylinder

The following solutions are prepared:

0.01 M EDTA solution: EDTA disodium dehydrate (0.9306 g) was weighted out into a 250 ml volumetric flask, dissolved in distilled water, and the volume was made upto the mark with distilled water, by shaking.

0.01 M $ZnSO_4$ solution as primary standard : 0.1614 g of zinc sulphate was weight out into a beaker, dissolved in dilute H_2SO_4, neutralized with NaOH solution, and transferred to a 100 ml volumetric flask. The volume was made up to the mark with distilled water, by shaking.

Buffer solution : 7g of ammonium chloride was mixed with 56.8 ml of concentrated ammonium hydroxide and the mixture made up to 250ml with distilled water.

Indicator : Eriochrome black-T

Procedure :

Standardization of EDTA solution :

10 mL of standard $ZnSO_4$ solution was pipette out into a 250 mL conical flask. To this solution, 5mL of (NH_4Cl + NH_4OH) buffer solution was added. This solution was warmed to about 40^0C. The EBT indicator 5 drops was added, and the mixture shaken to obtain a wine - red colour. This solution was titrated with EDTA solution until the wine-red colour changed to blue. The titration was repeated to get concurrent values.

Titration of milk solution with 0.01 M EDTA solution :

10 mL of the milk solution was pipette out into a 250 mL conical flask. To this solution, 5mL of (NH_4Cl+NH_4OH) buffer solution was added. This solution was warmed about 40^0C. The EBT indicator was added, and the mixture shaken to obtain a wine - red colour. The solution was titrated with EDTA solution (0.01M) until the wine-red colour changed to blue. The titration was repeated to get concurrent values.

Amount of calcium (in gm) = Molarity of calcium × molecular weight of calcium

Reference

1. https://scs.niser.ac.in/labhandouts/C142-Exp-7” estimation of calcium milk from poder through EDTA complexometry.

CHAPTER 4

RESULT AND DISCUSSION (GRAPH)

Result

Calcium content of various milk samples was determined without subjecting the milk samples to boiling. 33samples (11 brand in triplicate) were tested. The observation are summarised in Table 1 and Chart 1. This values indicate that the highest Calcium content was found in Goat milk (0.85 g/l), followed by cow milk (0.79 g/l). In buffalo milk, observed value was found (0.75 g/l). Among commercially available samples Amul, Dinshaw, Haldiram, Gowardhan, Mother's dairy, and milk powder recorded lower calcium content as compare to Goat, buffalo and cow milk respectively.

Sr. No.	Sample	Amount of Calcium content g/10ml			Amount of calcium (g/ml)
		I	II	III	
1	Amul Milk	0.56	0.57	0.57	0.57
2	Dinshaw Milk	0.61	0.63	0.63	0.63
3	Haldira milk	0.78	0.78	0.79	0.78
4	Prabhat Milk	0.33	0.31	0.31	0.31
5	Cow Milk	0.82	0.79	0.79	0.79
6	Buffalo Milk	0.75	0.75	0.78	0.75
7	Goat Milk	0.87	0.85	0.85	0.85
8	Mothers Dairy Milk	0.66	0.64	0.64	0.64
9	Gowardhan Milk	0.29	0.29	0.27	0.29
10	Nestle Milk Powder	0.17	0.14	0.14	0.14
11	Britania Milk Powder	0.11	0.11	0.11	0.11

DISCUSSION

One of the most vital components of milk for human nutrition is Calcium which is needed for tissue and bone development while deficiency results in slow growth, deprived appetite and rickets. In the present study, Calcium content in goat milk has been found to be higher than that of in buffalo, cow and packaged milk. According to Belewu (2002) (1) good milk has higher Calcium content because superiority of good milk lies in its higher content of digestible Calcium. Lowest Calcium content was found in commercially available milk powder. Similar observations have been recorded by Imran (2008) where buffalo milk recorded maximum Calcium content. In the present study, value of Calcium content is higher than previous study (Imran et. Al, 2008). Studies of Imran (2008) (2) indicate the levels of Ca^{2+} in Goat and Baffalo milk as 1.993 gm/l and 0.644gm/l respectively. These differences may have been resulted due to variation in geographical location, environment etc. Feeding and nutritional status of the animal may also strongly contribute to the variation among the animals. Variation can also be explained by breed differences and the differences in stage of lactation as reported by Islam et al. (2014) (3).

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